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Evaluation of COVID-19 Vertical Maternal Transmission with Respect to Foetal Visceral Maturation

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Novel corona virus infection could be transmitted from an infected pregnant woman to her infant, a process termed vertical infection. The study aimed to find the relationship between the SARSCoV-2 virus invasion and infection occurring during pregnancy and the nature and extent of impaired organ maturation in foetus. An observational, descriptive pilot study was conducted with 4 still born foetuses and their thymus, spleen, lungs and kidneys were processed. The microscopic examinations were done to evaluate the degree of visceral impairment. Our results revealed that mothers affected with COVID-19 infection during the first trimester of pregnancy exhibited impaired maturation for foetus of 33 weeks and 37 weeks compared to the viral disease during the last trimester of pregnancy of foetus of 38 weeks and 27 weeks. The study helps us to find out the possible mechanisms behind it as well as the nature of the maternal and foetal response to COVID-19 occurring during pregnancy.

Key words: Maternal infection, SARS-CoV-2, impaired fetal organ maturation, vertical transmission

Introduction

Following the identification of an outbreak of novel coronavirus infection (SARS-CoV-2), in Wuhan, China, in December 2019, there was concern for the potential

effects of the illness on pregnant women which impair the visceral maturation of foetus by vertical infection [3, 22]. In recent times, the histology of the embryological development of various organs has been studied and helps to correlate with the gestational age (GA). Histological examination of fetal viscera show marked qualitative or quantitative changes during development and marked as relevant [18]. Thymus is a lymphoepithelial organ and the key regulator of cellular immunity of the body [1]. Meanwhile, largest accumulation of lymphoid tissues in the body in spleen serves as defense against microorganisms that penetrate the circulation [10]. The human kidney develops through a complex process termed as 'branching' morphogenesis between 22 and 36 gestational weeks. This creates a radial glomerular pattern [8]. The histology of lung can also be a reliable parameter, [11] and radial alveolar count requires a pleural section parallel to the bronchiolar tree. The radial alveolar count described by Emery and Mithal is the number of alveoli crossed by an imaginary straight line drawn from the center of a terminal bronchiole to the nearest pleural surface [13]. Gestational age was calculated based on maternal data (last menses: Naegele's rule) [15]. Ultrasound data were obtained from the medical records department and possible genetic abnormalities were excluded and causes of death were identified as in utero death or spontaneous.

In viral defense mechanisms during pregnancy, syncytiotrophoblast of placenta possesses high rates of basal autophagy [5, 7]. This has critical role in the maternal-fetal interface and the destruction of the trophoblast may serve as a potential mechanism for a pathogenic virus to penetrate the chorionic villi. This reaches the fetal circulation which results in a programmed cell death [7]. The information on the effects of SARS-CoV-2 infection in pregnancy is limited [3, 12]. Thymic microstructure has specialized anatomical organization which is directly proportional to their function [4]. The medulla represents a site where each single positive thymocytes accumulate prior to their exit into the periphery. Subsets of medullary thymic epithelial cells are involved in multiple aspects of T-cell development and thymic migration. Medullary heterogeneity provides a better understanding of the mechanisms controlling α and β T-cell development especially in innate and adaptive immune systems [6, 17]. In severe chronic forms of viral diseases splenic tissue exhibits white pulp atrophy, to the degree that secondary lymphoid follicles completely disappear [9]. Changes in the structure of the spleen with splenic or lymphoid stromal hyperplasia, may be followed by lymphoid atrophy and disorganized compartments of the spleen. The development of human kidney is a complex process. The definitive and morphologically distinctive sequential developmental pattern of the glomerulus, commencing as early as 7^{th}–8^{th} week of gestation and continuing up to 35–36^{th} week of gestation, makes the fetal kidneys excellent viscera for estimation of period of gestation [8]. The histology of lung can also be a reliable parameter and radial alveolar count requires a pleural section parallel to the bronchiolar tree [11, 13]. Development of lung is a continuous process till 8 years and by 20^{th} week there is differentiation of the type 1 pneumocytes. Pneumocytes when infected early, can led to recruitment of leukocytes into the pulmonary interstitium, production of pro-inflammatory cytokines, injury to

parenchymal cells, collapse of the alveolar space which compromise of gas exchange and could cause hypercapnia [16].Thymus and spleen completes its embryological development by first trimester whereas kidney by third trimester and lung by 8th year of life, so that we can understand the effect of viral load during the development of these organs despite of the period of SARS-CoV-2 infection in pregnancy. Histological examination of the kidney has the advantage of revealing clearly visible structural changes that are still recognizable in cases of advanced necrosis, which is frequently encountered in forensic practice. The lung was also a reliable parameter in cases of putrefactive changes due to infections. Considering other organs for estimating gestational age, reports in the literature have shown that many pathological conditions can modify the histological examination e.g. pancreas, genital organs, and liver [18]. The case series aimed to find the relationship between the SARSCoV-2 virus invasion and infection occurring during pregnancy and the nature and extent of impaired organ maturation in foetus. Objectives under consideration were to characterize the foetal organ pathology findings in neonates infected by transplacental transmission arising from maternal infection with COVID-19 and to identify pathological risk factors for foetal infection.

Case series

Four different histological samples namely, thymus, spleen, lungs and kidneys were processed. All samples were fixed in 10% formalin and embedded in paraffin; 5 μm sections were stained with hematoxylin-eosin for light microscopy. The microscopic examinations were done to evaluate the degree of visceral maturation based on knowledge of the developmental chronology of foetal tissue. The GA with histological development of the foetal thymus maturation and spleen were noted. For the kidneys, gestational age were estimated by counting the rows of glomeruli between two well-oriented columns of Bertin running from the arcuate artery to the nephrogenic zone or with sequential development of glomerulus by counting the average radial glomerular count in cortical zones. Lung development was determined by the different stages of development based on the maturation.

History of COVID-19 infection was during the first trimester of pregnancy for mothers of foetus number 1 aged 33 weeks (wks) and foetus number 4 aged 37 wks whereas it was during the last trimester for mothers of foetus number 2 aged 38 wks and foetus number 3 aged 27 wks. We took written informed consent.

Foetus No.1 of 33 weeks weighted 1.26 kg had the following features that the kidney section studied from first image showed radial glomerular count (RGC) of 15. Second image showed 17 and third image showed single glomeruli. Thymus section show thymic parenchyma with well-formed trabeculae. Parenchyma composed of loosely arranged mesenchymal cells and lymphocytes. Cortex and medulla cannot be distinguished. No Hassall's corpuscles seen. Spleen section showed predominantly red pulp and indistinct white pulp. No trabeculae or capsule noted. Lung section showed extensive area of haemorrhage with small foci and congested blood vessels,

which are suggestive of intravascular coagulation. Lungs are in saccular stage of lung development (**Fig. 1**)

Foetus No.2 of 38 weeks weighed 2.5 kg had the following features that the kidney section studied from first image showed RGC of 2. Second image show 27 and third image is not showing any glomeruli. Thymus had well-form ed capsule and trabeculae dividing into lobules composed of lymphocytes. Two Hassall's corpuscles were noted. Spleen showed well-formed trabeculae with red pulp. White pulps not seen in the image provided. Lungs were in alveolar stage of lung development. There was no hyaline membrane in alveoli (**Fig. 1**).

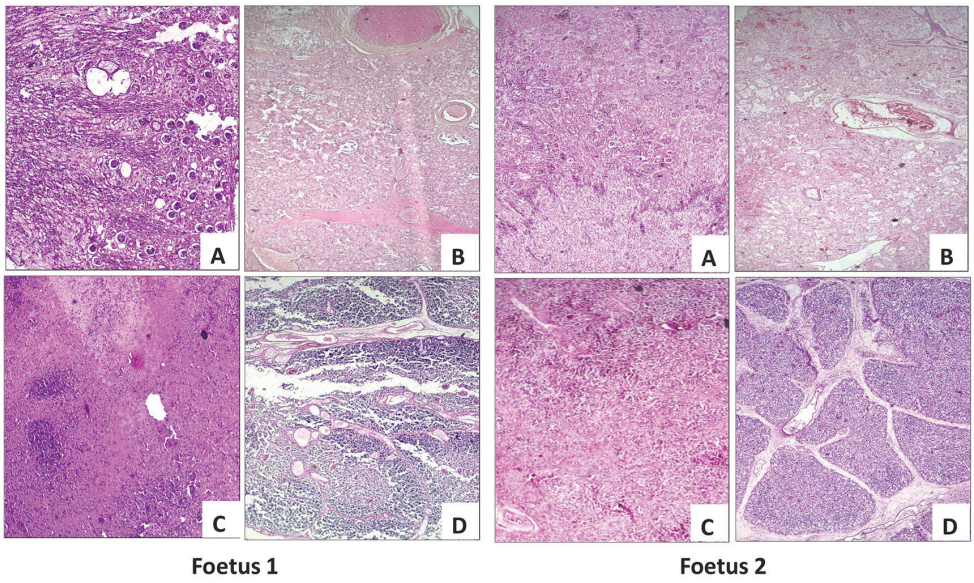

Fig. 1. Foetus 1(33 Wks) and Foetus 2 (38 Wks) : A-Kidney (Kidney average RGC is 11 corresponding to 24 weeks of gestation of foetus 1 and Kidney RGC is 14.5 corresponding to 28 weeks of gestation of foetus 2)**, B-Lung (**Lung shows primitive alveoli form corresponding to 26-32 weeks of gestation of foetus 1and Lung shows primitive alveoli form corresponds to 32-38 weeks of gestation of foetus 2)**, C-Spleen** (Spleen group IV corresponding to 18-24 weeks for both foetus 1 and 2)**, D-Thymus** (Thymus group IV corresponding to 18-24 weeks of foetus 1 and Thymus groups corresponds to 25-38(group V) weeks of gestation of foetus 2).

Foetus No.3 of 27 weeks weighted 900g had the following features that the kidney section studied from first image showed RGC of 17. Second image RGC is 3. Thymus had thin capsule and trabeculae dividing lymphocytes into lobules. No Hassall's corpuscles were noted. Spleen showed trabeculae with red pulp. No white pulp seen. Lungs are in saccular stage of lung development (**Fig. 2**).

Foetus No.4 of 37 weeks weighted 2 kg had the following features that the kidney section studied from first image showed RGC of 27 and second image showed 0. Thymus had thin capsule and trabeculae dividing lymphocytes into lobules. No Hassall's corpuscles were noted. Spleen showed trabeculae with red pulp. No white

pulp seen. Lung section showed extensive area of haemorrhage with small foci and congested blood vessels, which are suggestive of intravascular coagulation. Lungs were in saccular stage of lung development (**Fig. 2**).

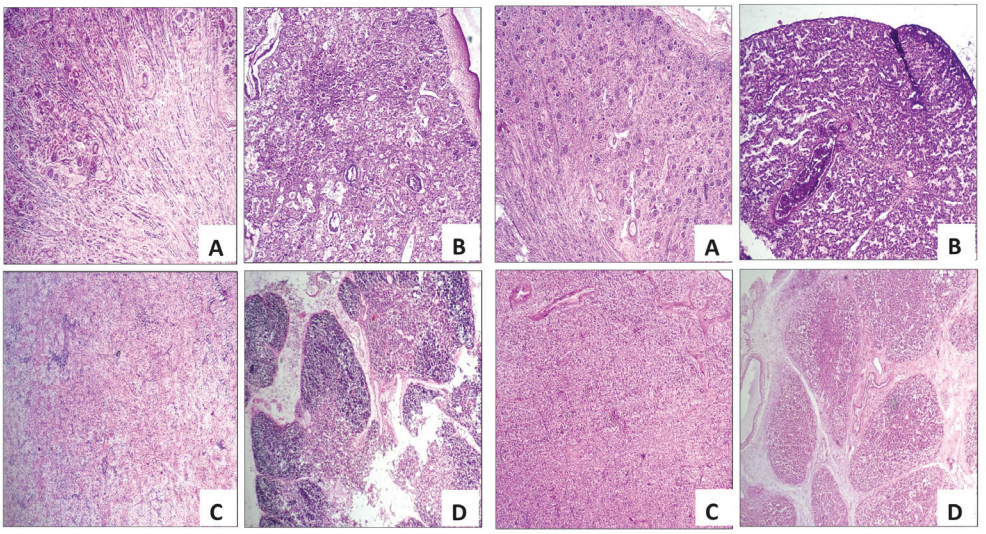

Fig. 2. Foetus 3 (27 Wks) and Foetus 4 (37 Wks) : A-Kidney (Kidney average RGC is 10 corresponding to 22 weeks of gestation of foetus 3 and Kidney RGC is 13.5 corresponding to 27 weeks of gestation of foetus 4)**, B-Lung (**Lung shows primitive alveoli form corresponding to 26-32 weeks of gestation for both foetus 3 and 4)**, C-Spleen** (Spleen group IV corresponding to 18-24 weeks for both foetus 3 and 4)**, D-Thymus** (Thymus group IV corresponding to 18-24 weeks for both foetus 3 and 4).

Discussion

Congenital transmission of SARS-CoV-2 was detected during the first trimester in the placental cells, amniotic fluid and also in the fetal membrane. However, the evidences were inadequate to establish foetal tissue involvement due to lack of samples for autopsy to study the virus particles [20]. In the present case series, we had two still birth foetus from the mothers diagnosed with SARS-CoV-2 during the first trimester of pregnancy. All organs which were studied showed delayed maturation which implies that the disease during the first trimester is causing more developmental issues on foetus as it is a period of organogenesis. The hypoxia induced on mothers due to the disease can also have an impact on foetoplacental circulation. However an extensive study with more number of samples is needed to draw a conclusion. Fetal tissues like the liver, heart, lungs and hematopoietic cells also express ACE2 which indicates that the presence of the virus in the amniotic fluid will cause the fetal infection [14]. In the present case series we had two fetuses obtained from mothers

who were affected by SARS-CoV-2 during the last trimester. It has been noticed that lung and thymus development appeared to be normal when the infection was in the last trimester, where spleen and kidney development was impaired irrespective of trimester of pregnancy and viral infection.

The thymus is an organ commonly targeted by infectious pathogens such as viruses, bacteria, and fungi. Alterations of proliferation, secretion, migration, differentiation and death of thymocytes can be induced by these infections due to phenotypic and functional changes within the thymus. The behavior of mature, peripheral T-lymphocytes can be equally affected [2]. In the present case series, two samples were from mother diagnosed with SARS-CoV-2 during the first trimester of pregnancy showed delayed maturation of thymus, which implies that the disease during the first trimester is causing more developmental issues on foetus as it is a period of organogenesis.

Irrespective of the trimester all cases had impaired growth in the spleen in the present case series. Splenic tissue of human fetuses develops at 14^{th} to 24^{th} week of gestation. The function of spleen in the foetus is hematopoietic and it continues from the fetal period till the child birth. So the red and white pulp attains maturation throughout the embryonic period though the maturation is complete. The viruses must have caused impaired function of the splenic cords due to hypoxia induced to the mother. Mild to moderate disorganization of the white pulp with indistinct regions and severe congestion and hemorrhage, and proliferation of megakaryocytes of red pulp was observed in the case series. An obvious distinction between white and red pulp is not always proper and several plasma cell aggregates replace the populations of normal resident cell of the red pulp observed [19].

Kidneys have the advantage of revealing clearly visible structural changes. The count of the glomerular zone extends from the top of the superficial definitive glomerulus to the bottom of the deepest glomerulus, at the junction with the medulla which is still recognizable in cases of advanced necrosis and frequently encountered in forensic practice [21]. In the case series all the samples have delayed and impaired glomerulogenesis. This indicates SARS-CoV-2 can cross the placental barrier as the viral RNA was detected in the amniotic fluid and the S proteins were detected in the fetal membrane [20].

Development of lung is a continuous process till 8 years and by 20^{th} week there is differentiation of the type 1 pneumocytes. The histopathological changes we observed in the infected lungs of K18-hACE2 mice correlate with the impaired pulmonary function [16]. It has been noticed in the case series that lung development appeared to be normal when the infection was in the last trimester. The pseudoglandular period in which most of the lung elements develop except alveoli must have likely to be affected by the viral disease which resulted in impaired growth of lungs of foetus born from mothers affected with COVID-19 infection during the first trimester.

Conclusion

The case series outlooks the impaired maturation of foetal vital viscera as can be related to SARS-CoV-2 virus invasion and infection during the pregnancy. This can prove the vertical transmission of infection through placenta and impaired organ development irrespective of the trimester of COVID-19 infection. The report helps us to find out the possible mechanisms behind it as well as the nature of the maternal and fetal response to COVID-19 infection occurring during pregnancy.

Limitations

We couldn't perform a placental or viseral immunohistochemical study with SARS-CoV-2 nucleocapsid-specific or cytokeratin-7 specific monoclonal antibody to detect SARS-CoV-2 antigen.

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